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List of abbreviations

CA concentration addition

CFST counterfactual susceptibility types

DRC dose-response curve

EC_{01} effective concentration causing 1% of maximal response

ES effect summation

IA independent action

IC interaction contrast

NOEC no observed effect concentration

RDA risk difference additivity

TEF Toxic Equivalency Factor

Abstract

Background: Epidemiologists and toxicologists face similar problems when assessing interactions between exposures, yet they approach the question very differently. The epidemiologic definition of interaction leads to the additivity of risk differences (RDA) as the fundamental criterion for causal inference about biological interactions. Toxicologists define interaction as departure from a model based on mode of action: Concentration addition (CA, for similarly-acting compounds) or independent action (IA, for compounds that act differently).

Objectives: We compare and contrast theoretical frameworks for interaction in the two fields.

Methods: The same simple thought experiment has been used in both fields to develop the definition of non-interaction, with nearly opposite interpretations. In epidemiology, the "sham combination" leads to a requirement that non-interactive dose-response curves be linear. In toxicology, it results in the model of concentration addition. We apply epidemiologic tools to mathematical models of concentration-additive combinations to evaluate their utility.

Results: RDA is equivalent to CA only for linear dose-response curves. Simple models demonstrate that concentration-additive combinations can result in strong synergy or antagonism in the epidemiologic framework at even the lowest exposure levels. For combinations acting through non-similar pathways, RDA approximates independent action at low effect levels.

Conclusions: While epidemiologists have argued for a single logically consistent definition of interaction, the toxicologic perspective would consider this approach less biologically informative than a comparison with CA or IA. We suggest methods for analysis of concentration-additive epidemiologic data. The two fields can learn a great deal about interaction from each other.

Introduction

Environmental exposures often consist of exposure to multiple agents. A complete understanding of interactive effects would require both the ability to identify unexpected interactions, in order to improve our biological understanding of mechanisms, and an ability to predict combination effects, in order to improve risk assessment and public health decision-making.

Both epidemiologists and toxicologists approach interaction assessment by defining a non-interactive model, departures from which are then considered interactive (e.g., synergistic or antagonistic). The choice of model—the definition of non-interaction—is the critical first step, since appropriate models will lead to more biologically informative conclusions regarding synergistic or antagonistic action. The definition of non-interaction in the two fields, however, proceeds very differently.

Epidemiologists have divided interactions into several "contexts", referring to statistical, biological, public health, and individual interactions (Blot and Day 1979; Rothman et al. 1980). Most methods that epidemiologists and biostatisticians use to assess interaction are explicitly statistical in nature, and should not, as these authors point out, be expected to provide information about biologic mechanism of action. For inference about causal mechanisms, epidemiologists have often relied on a single criterion, the additivity of risk differences, considering deviation from risk difference additivity to be the single appropriate metric for examination of biological interaction (Ahlbom and Alfredsson 2005). By contrast, toxicologists (and pharmacologists) have developed several models for biological non-interaction, each of which is rooted in a simple assumption about mode of action.

Although the word "interaction" is used in both fields, these divergent approaches have led to dramatically different understandings of its meaning. Here we explore these differences, and demonstrate that some combinations considered non-interactive by toxicologists are likely to meet the criteria for interaction in an epidemiologic analysis.

Epidemiologic analysis of interaction

Epidemiologic theory of biological interaction is rooted in counterfactual models, which describe all possible responses of individuals to different patterns of exposure. Both the counterfactual susceptibility types model (CFST) and the sufficient–component causes ("causal pies") model are deterministic descriptions of binary outcomes due to dichotomous exposures, intended to define the range of possible biological outcomes without reference to any specific mechanism (Rothman et al. 2008).

The CFST model describes all possible ways by which individuals of different counterfactual susceptibility types could react to a binary exposure (Greenland and Poole 1988); the model is usually considered deterministic, where each individual always responds according to their type. Given a single binary exposure X with a binary outcome, there are four possible types for the exposures x=0 (unexposed) and x=1 (exposed) (Table 1) (Greenland and Robins 1986). From this description of the possible individual responses, one can construct the response in the population as a whole. Taking p_1 through p_4 to represent the proportion of each type in the population, one calculates risks in the population by simply adding proportions. For example, the risk in the population when exposed to X is simply p_1+p_2 (since types 3 and 4 do not have the outcome and thus do not contribute to the risk).

The model is easily extended to two exposures (Greenland and Poole 1988). Having listed all possible response types for exposures X and Z (Table 2), one may describe a non-interactive population by eliminating the interactive types from the model (i.e., setting the proportion of those types in the population to zero). The interactive types were identified by Miettinen (1982) and clarified by Greenland and Poole (1988); many correspond to intuitive notions of synergy or antagonism. In the words of Rothman et al. (2008), "The defining feature of these 10 interaction types is that we cannot say what the effect of X will be... unless we know that person's value for Z". This definition depends on the interdependence of action of causal factors; indeed, some authors refer to non-interaction more specifically as "non-interdependence" (Greenland and Poole 1988), and some papers use the terms interchangeably (Greenland 1993).

Applying this definition, individuals of type 4 will always have the outcome when z=1, regardless of the value of X; for this individual, Z is causal and X ineffective. This is a non-interdependent (i.e., epidemiologically non-interactive) type, since one can predict the effect of Z without knowledge of the status of exposure to X. By contrast, an individual of type 8 responds only to the combined exposure X+Z. X and Z are thus interdependent in this type of individual: Without knowing the individual's Z exposure, one cannot predict the result of an exposure due to X. This corresponds to an intuitive definition of synergy, where both exposures are required to produce an effect.

Eliminating interdependent types leaves only types 1, 4, 6, 11, 13, and 16 in our population. Risks under the various exposure scenarios are designated as R_{XZ} (e.g., R_{10} is the risk in a population exposed to X (x=1) but not to Z (z=0)). Writing down risks in the four possible exposure combinations, and rearranging, one obtains a simple equation for non-interdependence (Rothman et al. 2008):

$$(R_{11} - R_{00}) = (R_{10} - R_{00}) + (R_{01} - R_{00}) \quad [1]$$

The risk difference due to the joint exposure is simply the sum of the risk differences due to the individual exposures. Since Equation 1 was derived using only the non-interdependent types, a departure must imply the presence of interdependent types. Thus, additivity of the risk differences (RDA) is a criterion for non-interdependence. It is a necessary but not sufficient criterion, however, since interdependent types may occur in a population in such a way as to satisfy the RDA equation (Rothman et al. 2008). Therefore, deviation from RDA indicates interaction, but satisfying RDA does not prove lack of interaction. (We assume, here and in the following discussion, that bias and confounding are absent.)

The RDA criterion derives from counterfactual models describing biological responses without depending on any specific mechanism. Therefore, deviation from RDA is seen as the fundamental criterion for biological interaction in epidemiology: "an unambiguous definition of biologic interaction" (Rothman 2002). Although derived from binary models, the RDA criterion is also used for continuous epidemiologic exposures including cholesterol, hypertension, age, coffee consumption, smoking, and others; these continuous values are typically categorized before RDA is applied (e.g., Hallqvist et al. 1996).

In practice, many of the epidemiologic parameters available for assessing interaction — including Koopman's "interaction contrast" (IC), Rothman's S index, and Walker's attributable fraction due to interaction I(AxB) — are derived from the RDA criterion (Koopman 1981; Rothman 1976; Walker 1981). Other authors have demonstrated how to use Cox and logistic regression to find departures from additivity on the risk difference scale, with the explicit goal of assessing biological interaction (e.g., Andersson et al. 2005).

The sham combination in epidemiology

It has long been recognized that shape of the dose-response curve (DRC) complicates interaction assessment when exposures are continuous (Greenland 1993). Rothman (1974) demonstrated an important implication of RDA for this situation in an interesting thought experiment. Consider the construction of a response from a series of non-interacting "distinct causes" made up of successive doses of the same agent (assume that the outcome describes the risk in a population). Starting with an initial dose X of 1 unit, producing a risk R_{10}, add an equal second dose, Z. Since the agents and doses are identical, $R_{10}=R_{01}$. With no background risk, the RDA equation yields

$$R_{11} = R_{10} + R_{01} = 2\ R_{10} \qquad [2]$$

The "doubly exposed", those receiving two units of dose, have twice the risk of the singly exposed. For this to hold regardless of choice of dose requires that the dose-response relationship be linear: The linear DRC represents non-interactivity (non-interdependence) under the RDA definition. Any nonlinear dose-response curve will exhibit interdependence (synergy or antagonism), and may, in fact, exhibit synergy in one dose range and antagonism in another. Intuitively, when the dose-response relationship is nonlinear we require knowledge of the initial dose—the position along the DRC—in order to predict the additional effect of the subsequent dose. The sham combination is a thought experiment, not a method used by epidemiologists to examine the shapes of dose-response curves. Nevertheless, these conclusions logically follow from the use of non-interdependence to derive RDA.

Toxicologic analysis of interaction

Unlike epidemiologists, toxicologists' ideas about interaction do not start with counterfactual models (i.e., describing responses of different types of individuals). Because modern toxicologists can control both the exposures and randomization of nearly identical subjects, they are typically little concerned with confounding or bias; from an epidemiologic point of view, they are essentially studying one type of individual. While counterfactual models could be constructed for studies of diverse animal populations, they would require a large number of types to support the continuous exposures (and, often, continuous outcomes) of interest in toxicology. For example, some older toxicology studies used a "quantal" model describing binary outcomes which occur when an individual's tolerance for an exposure is exceeded (Finney 1971). This model is closely related to the CFST model, since it deterministically predicts each individual's outcome for any exposure condition once we know the individual's threshold. (It could be expanded to include "doomed" individuals that develop disease with no exposure, or "immune" individuals having an infinite threshold.) By analogy with the CFST model, each possible exposure threshold is a different susceptibility type, and so the continuous quantal model contains an infinite number of possible types. Instead of using counterfactual models, however, toxicologists begin the study of interaction with dose-response curves.

Like epidemiologists, toxicologists define "interaction" as a departure from a non-interaction criterion, usually called in toxicology the "null model". Unlike epidemiologists, toxicologists use several different null models. Starting with a null model, and the dose-response curves of each agent given individually, one may construct the expected (non-interactive) response to a combination exposure. This approach dates back to Bliss (1939), who defined

"synergistic action" as any case where "the effectiveness of the mixture cannot be assessed from that of the individual ingredients". For a toxicologist, a joint effect is considered non-interactive if it follows a simple biological expectation which can be predicted from the responses of the individual agents. (Null models are often said to describe "additive" effects; we avoid that term due to its potential for confusion.) These null models are also used in risk assessment to predict expected effects when more specific mechanistic details are not known (US EPA 2000).

The simplest null model, commonly used by toxicologists by implication and often without justification, is effect summation (ES):

$$f(x,z) = f(x,0) + f(0,z) \qquad [3]$$

where $f(x,z)$ describes the joint response for exposure to agents x and z; $f(x,0)$ and $f(0,z)$ describe the dose response curve for each agent individually. Whether this definition includes a background response is not usually made explicit; for a more precise definition we might subtract the background effect $f(0,0)$ from each term,

$$f(x,z) - f(0,0) = f(x,0) - f(0,0) + f(0,z) - f(0,0) \qquad [4]$$

The epidemiologic RDA criterion (Equation 1) is a special case of (4) when the exposures are dichotomous.

The sham combination in toxicology

Effect summation may be intuitive—many toxicologists use it implicitly and uncritically—but it is generally considered insufficient. The major toxicologic argument against it is the "sham combination", a thought experiment essentially identical to the one described by Rothman, but interpreted very differently (Berenbaum 1989).

Consider a "combination" of two exposures A and B, which actually consist of the same toxic agent. We take $f(.)$ as describing the causal effect above the background. Effect summation (3) requires

$$f(A, B) = f(A, 0) + f(0, B) \quad [5]$$

but since B is identical with A, this is equivalent to

$$f(A+B) = f(A) + f(B) \quad [6]$$

This last condition is met only if the dose-response curve is linear in A. For example, if A=B, then $f(A+B) = f(2A) = 2\,f(A)$.

What happens if the DRC is not linear? Suppose it increases more rapidly than a linear response (Figure 1). With "sham" doses A=0.4 units, and B=0.6 units of the same agent, the effect of the combination dose $f(A+B) = f(0.4+0.6) = f(1.0)$ is much greater than the sum of the individual responses $f(A) + f(B)$.

The toxicologic sham combination yields the same result we saw in Rothman's thought experiment: $f(A, B) = f(A, 0) + f(0, B)$ only if $f(.)$ is linear; that is, effect summation holds only for linear dose-response. Toxicologists do not consider non-linearity to have special biological significance, but consider the dose-response curve of an agent to be a property of that agent (more precisely, a property of the agent working on a particular tissue or system). From a mechanistic point of view, many nonlinear DRCs can be modeled by simple biological

mechanisms (e.g., receptor filling) that toxicologists do not consider "interactive" in any important biological sense. Indeed, some toxicologists state that "the conclusion that an agent interacts with itself in a synergistic way is absurd" (Kortenkamp and Altenburger 1998). (There may be interesting exceptions, however. For example, the structural change in hemoglobin that results from allosteric binding of oxygen increases the availability of other binding sites to subsequent oxygen molecules. This is often referred to as "cooperativity".)

If one considers different doses of an agent as not synergizing with (or antagonizing) each other, and the nonlinear dose-response curve is therefore non-interactive, then the sham combination itself must be the non-interactive condition. This is the basis for the null model Bliss called "simple similar action", now called "concentration addition" (CA) or "dose addition".

The CA model is most easily derived for the case of joint exposure to two agents, A and B, which are not identical but which have parallel dose-response curves differing only by a factor of potency, that is, only in the amount of the agent required to produce the same effect. In that case, one can consider A as being a dilution of B by some factor γ, such that $f_A(\mathrm{A}) = f_B(\gamma\,\mathrm{A})$. By the same logic, one may substitute for the joint effect $f_{AB}(\mathrm{A,B}) = f_B(\gamma\,\mathrm{A+B})$. Consider a specific response level E, which is caused by either doses A_E or dose B_E when given independently, i.e., $E = f_A(\mathrm{A_E}) = f_B(\mathrm{B_E})$. It can easily be shown that any combination dose (A,B) will also cause E if it satisfies

$$1 = \frac{A}{A_E} + \frac{B}{B_E} \qquad [7]$$

Equation 7 defines the null model of concentration addition. (The non-interactive sham combination is the special case where A is identical to B.) Because this derivation assumes that

exposures A and B can be substituted for one another in proportion to their potencies, CA is usually considered appropriate for agents that act via a "similar" mechanism (US EPA 2000), although there has been considerable discussion as to the meaning of "similar" (Borgert et al. 2004). In assuming that A was a dilution of B, this derivation followed the Toxic Equivalency Factor (TEF) model, a special case of CA where the relative potency γ is constant for all effect levels. This is the best-known implementation of CA, commonly used for assessing and predicting non-interactive effects of combinations of dioxin-like agents (Van den Berg et al. 2006). However, the assumption of a constant value of $\gamma = B_E/A_E$ is not a requirement of the CA definition; more generally, γ can vary with effect level E if A and B have non-parallel DRCs (Howard and Webster 2009).

Concentration addition has rarely been mentioned by epidemiologists as a method for analyzing interaction (Cornfield 1975; Thomas and Whittemore 1988), yet it is widely used by toxicologists, particularly in the TEF form. Just as we expressed the total effect of the joint exposure (A,B) in terms of an isoeffective dose of B given by γ A+B, the TEF method allows toxicologists to express a mixture of similarly-acting chlorinated dioxins, dibenzofurans, and polychlorinated biphenyls by a single equipotent dose.

Another important null model used by toxicologists is Bliss's "independent joint action" (IA). This model describes causal action in stochastic terms, where the joint outcome is the probabilistic sum,

$$P(A+B) = P(A) + P(B) - P(A)P(B) \qquad [8]$$

IA depends on the statistical independence of the two exposures; the same model has been discussed by epidemiologists (Rothman 1974; Weinberg 1986). In the case of low risks, when one may ignore the product term, it is approximated by RDA (Rothman 1974). Similarly, effect

summation is sometimes used by toxicologists and by risk assessors as a low-risk approximation to independent action (US EPA 2000).

These three null models—IA, CA and ES—are those most commonly used in toxicology. For toxicologists, CA and IA have firm biological foundations based on assumptions about mode of action; ES does not. In epidemiology, there is still discussion about the appropriate measure for interaction assessment, including the choice between the additive (risk difference) or multiplicative (risk ratio) scales (Weinberg 2012). Concentration addition, however, occurs on neither of these scales. Instead of adding or multiplying the effects of individual agents, CA involves addition of weighted exposures (i.e., isoeffective doses). CA's inherent dependence on the shape of the dose-response curve means that a straightforward mathematical combination (e.g., addition or multiplication) of the outcomes (i.e., the joint effects or risks) will not be an adequate description of joint action for a concentration-additive combination unless the dose-response is linear.

Concentration Addition and Risk Difference Additivity

We have seen that risk difference additivity is a special case of the toxicologic model of effect summation. Here we examine its usefulness in evaluating concentration-additive exposures.

Counterfactual type 2 is a particularly interesting example: this is the only type in which each exposure is effective individually and the joint exposure is also effective. From the epidemiologic perspective, it is interdependent (Greenland and Poole 1988), since for the binary outcome of the CFST model, each exposure causes an effect only if the other is absent. While

logical, toxicologists would not consider this conclusion informative in the intended biological sense.

For example, one of the most common DRCs in toxicology is the Hill function,

$$f_A(A) = \frac{A^n}{A^n + K_A^n} \qquad [9]$$

where K_A is the dose producing half-maximal effect—a measure of potency—and n is a slope parameter. When n=1, the dose-response curve for a single agent increases less rapidly than linear (Figure 2), and a sham substitution would be considered antagonistic using ES (or RDA).

Consider a TEF model in which B has a relative potency γ compared with A; that is, $f_A(\mathrm{A}) = f_B(\gamma\ \mathrm{A})$. The joint effect for two (n=1) agents is given by:

$$f_{AB}(A,B) = f_B(\gamma A + B) = \frac{(\gamma A + B)}{(\gamma A + B) + K_B} \qquad [10]$$

Any given effect level between 0 and 1 can be achieved (or any tolerance exceeded) using A alone, B alone, or a combination of A and B. (The maximum possible effect of 1 is reached in the limit of large dose.) We take K_A=2 and K_B=1, so A is half as potent as B, and γ=0.5; setting the exposure for each agent to a large value compared with its own K, we calculate the responses to two dichotomous doses (Figure 3A).

Suppose that this thought experiment takes place in a population of identical individuals who, when subjected to this combination exposure, will suffer a binary health outcome above a response threshold of 0.8. The risks under these dichotomous exposure conditions are given by Figure 3B; each individual responds according to the type 2 pattern. This is an interdependent type, since the outcome of exposure to A differs in strata of B. (Alternatively, consider a population of identical responders for whom Equation 10 gives the probability of a binary

outcome; since the individuals are identical, the responses in Figure 3A directly describe the population-level risks, which we may then use to test for epidemiologic interaction. Application of the interaction contrast or Rothman's S index to these risks yields IC=-0.774 and S=0.54, both clearly indicating interaction relative to RDA.) Yet from a toxicologic perspective, the system is concentration additive and non-interactive. Furthermore, Equation 10 is consistent with saturation of a receptor system, not considered a case of biological interaction by toxicologists.

Now consider counterfactual type 8, where the outcome occurs only with exposure to both agents. From the epidemiologic perspective, this interdependent type is intuitively synergistic. We can, however, produce results consistent with this situation using a concentration-additive model. Consider a Hill function with n=2 (Figure 2); many estrogenic agents have a value of n between 2 and 3 (Silva et al. 2002). Applying the TEF model, the joint effect is

$$f_{AB}(A,B) = f_B(\gamma A + B) = \frac{(\gamma A + B)^2}{(\gamma A + B)^2 + K_B^2} \qquad [11]$$

Again taking K_A=2 and K_B=1, and choosing dichotomous doses for the "exposed" that are very low compared with K_A and K_B, we obtain Figure 4A. Suppose that this experiment takes place in identical individuals. For a binary outcome defined by a threshold of 0.1, the risks are 1 in the doubly exposed and 0 elsewhere (Figure 4B), following the pattern of type 8 (synergistic) individuals. (For the probabilistic interpretation of Figure 4A, application of IC or the S index shows strong synergy with respect to RDA: IC=0.049 and S=1.8.) Note that the choice of a different, much lower threshold in determining Figure 4B would produce results that appear characteristic of type 2 individuals. This change in results occurs because Figure 2 rises steeply at low doses and flattens at high doses. From a toxicologic point of view, there is nothing

remarkable about such a dose-response curve nor interactive about these results: The system is concentration additive.

Finally, we consider experimental evidence for such systems (Silva et al. 2004). These authors tested a mixture of eight estrogenic agents in a yeast culture assay, and found that the results followed the CA model. Importantly, each of these agents was exposed below its NOEC (No Observed Effect Concentration) or EC_{01} (the concentration producing 1% effect)—in either case, a dose producing a very low effect—yet the mixture produced a strong effect, larger by a factor of 20 than that predicted by effect summation. Using the criterion of effect summation, this is a clear case of synergy. But the results are not interactive under CA, since the latter model accurately predicts the joint effect from the individual components. Because each agent acts as a dilute form of estrogen, the small doses combine to cause a single effect through their common pathway.

Discussion

Risk difference additivity derives from counterfactual models of susceptibility types. The underlying definition used to eliminate interactive types is that of interdependence. Basing interaction on this underlying idea seems intuitive. In individuals of identical CFST type, if the effect of X is the same regardless of any simultaneous exposure to Z, then Z evidently has no effect on X: i.e., there is no dependence of the effect of X on the effect of Z (or vice versa).

Application of the RDA criterion to the sham substitution leads to the conclusion that a compound can synergize with or antagonize itself, or both, depending on the shape of the response and the specific doses evaluated. Under the epidemiologic definition, a nonlinear dose-response must be seen as interactive, a conclusion toxicologists do not consider biologically

insightful. Following Bliss, toxicologists instead define "interaction" as a synergistic or antagonistic departure from an expected joint effect. Risk assessors use the same approach, reserving "interaction" for departures from a model based on the action of individual components (US EPA 2000). This approach construes the dose-response curve itself—and thus the sham combination—as non-interactive. For similarly-acting compounds, the result is the CA null model.

The epidemiologic and toxicologic perspectives rely on different definitions of interaction. Neither definition can be said to be true or false; the question is whether they lead to useful results. From the toxicologic perspective, the epidemiologic RDA criterion may supply little biologically useful information. We have shown using simple biological models that results consistent with two counterfactual susceptibility types considered by epidemiologists to be interdependent—type 2 and type 8—may be non-interactive from the toxicologic perspective. Concentration-additive exposures, like those in our examples, must always be interdependent, since each agent acts by contributing to a single pathway. Rather than using non-interdependence, the toxicologic approach defines non-interactivity using null models based on general modes of action. Deviation from the null model then implies something unexpected about the underlying biology.

Epidemiology has the advantage of a single definition for judging interaction, rigorously and logically applied. There are three definitions for non-interaction in general use in toxicology, with one, effect summation, considered inappropriate by mixtures toxicologists. The other two—concentration addition and independent action—are used as null models for compounds with similar or different modes of action, respectively. This raises the question of what toxicologists mean by "similar", a question that is still debated. Despite this important issue, there has not

been a proliferation of toxicologic null models, each with its corresponding type of synergy or antagonism. Although more sophisticated models have sometimes been proposed (e.g., Rider and LeBlanc 2005), toxicologists and risk assessors have generally restricted themselves to the models, CA and IA, originally suggested by Bliss (US EPA 2000). Indeed, toxicology may be approaching a point suggested by epidemiologists almost thirty years ago: "Such classification [of biological models for interaction] could be a useful shorthand to describe categories of mechanisms, but only if such categories were widely and explicitly agreed upon in the scientific community" (Rothman et al. 1980).

Which approach for assessing interaction yields the most insight? Or will combinations of the epidemiologic and toxicologic approaches work better? One approach to answering these questions is to apply both sets of methods to appropriate epidemiologic (or toxicologic) datasets. A necessary foundation for comparing results or sharing ideas across the two fields is an understanding of the terminology and underlying concepts and methods of each. Practical questions in each field must be taken into account. For example, assessing interaction can be problematic in many real world epidemiologic studies given the primary concern with bias and confounding. Toxicology's narrow focus on identical individuals limits its applicability in real world situations; epidemiology's emphasis on counterfactual modeling might help toxicologists develop better methods for situations when all individuals are not identical. For example, if a population consists of two types of individuals who respond with different sensitivities to two chemical exposures, one can show that risks in the population as a whole need not be concentration additive, even if they are for each type of individual. Collaborative investigation of models like these may provide new insights for both fields.

For epidemiologists who wish to apply toxicologic methods for examining interaction, we provide these thoughts. When exposures operate through non-similar pathways, toxicologists consider independent action the best default model. If analysis is limited to low effect levels (when the product term in Equation 8 is negligible), or in the rare case of linear response, the toxicologic (IA) and epidemiologic (RDA) approaches reach the same conclusion. Current epidemiologic methods for examining interaction can then be used with little or no modification. For exposures operating through similar pathways, however, this is not the case. As we have seen, the departure of concentration additive exposures from risk difference additivity may be substantial even at the lowest doses. Fortunately, some of the approaches discussed below are already used by epidemiologists to construct exposure measures or analyze data, although not for examining interaction.

For exposures thought to act by similar mechanisms, and whose individual actions can be well characterized, a CA model can be used to predict the joint effects of combinations from mathematical functions describing the individual dose-response curves (e.g., Howard and Webster 2009). One can then construct non-interactive response surfaces for comparison with the data, testing for mode of action as well as for the presence of interactive effects (e.g., Howard et al. 2010).

More simply, similarly-acting exposures can be grouped into a single equipotent exposure using a TEF-like model (or a generalized concentration addition model). For dioxin-like agents, this is common practice (Van den Berg et al. 2006). Such a model is likely to be a productive route for examining exposure to estrogens and xenoestrogens, for example, or androgens and anti-androgens. More research is needed to generate the data needed for this approach and to test its applicability.

A third approach might employ a simple biological assay to estimate the combined activity—e.g., dioxin-like or estrogenic—of a complex mixture, e.g., human serum. The assay result could then be used as the exposure measure for the mixture. A combination of analytical chemistry and toxicologic analysis could then be used to determine the contribution of individual components and whether they explain the activity of the mixture.

In many cases, little detailed dose-response information is available for human populations. Here one might use the "method of isoboles": When the concentration of one agent is plotted against the concentration of the other, curves of constant joint effect (isoboles, or contours of the response surface) must be negatively sloped straight lines if the exposures are concentration additive. This simple visual analysis is amenable to use even with very small numbers of data points (Berenbaum 1989). Isobolographic analysis and related methods for response surface analysis (Greco 1995) may be useful when analyzing epidemiologic data, which is typically spaced irregularly in the concentration-concentration plane.

Interaction, and the terminology used to describe it, has long been a source of confusion and debate in both epidemiology and toxicology (Ahlbom and Alfredsson 2005; Könemann and Pieters 1996). We strongly believe that increased discussion and collaboration between the two fields will increase our understanding of interaction.

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Table 1. CFST model for a single exposure. Outcomes are given as 0 or 1 for the exposed (x=1) and unexposed (x=0) scenarios (Greenland and Robins 1986).

type	x=1	x=0	description
1	1	1	doomed
2	1	0	X causal
3	0	1	X preventive
4	0	0	immune

Table 2. CFST model for two exposures. Outcomes are given as 0 or 1. Interdependent types according to Greenland and Poole (1988) are marked with an asterisk (*); remaining non-interdependent types are shaded. See also Miettinen (1982).

type	x=1 z=1	x=0 z=1	x=1 z=0	x=0 z=0	description
1	1	1	1	1	doomed
2*	1	1	1	0	X causal, Z causal, joint causation by X+Z
3*	1	1	0	1	
4	1	1	0	0	Z causal, X ineffective
5*	1	0	1	1	
6	1	0	1	0	X causal, Z ineffective
7*	1	0	0	1	X preventive, Z preventive, X+Z antagonizes
8*	1	0	0	0	X+Z causal
9*	0	1	1	1	X+Z preventive
10*	0	1	1	0	X causal, Z causal, X+Z antagonizes
11	0	1	0	1	X preventive, Z ineffective
12*	0	1	0	0	
13	0	0	1	1	Z preventive, X ineffective
14*	0	0	1	0	
15*	0	0	0	1	X preventive, Z preventive, joint prevention by X+Z
16	0	0	0	0	immune

Figure Legends

Figure 1. The "sham combination" of two identical agents, in doses A=0.4 and B=0.6, yields a larger response than the sum of the individual effects if the dose-response curve has increasing slope.

Figure 2. Hill functions with slope parameters n=1 (solid line) and n=2 (dashed line). In each case, K=1.

Figure 3. Responses to the n=1 TEF joint exposure.

Figure 4. Responses to the n=2 TEF joint exposure

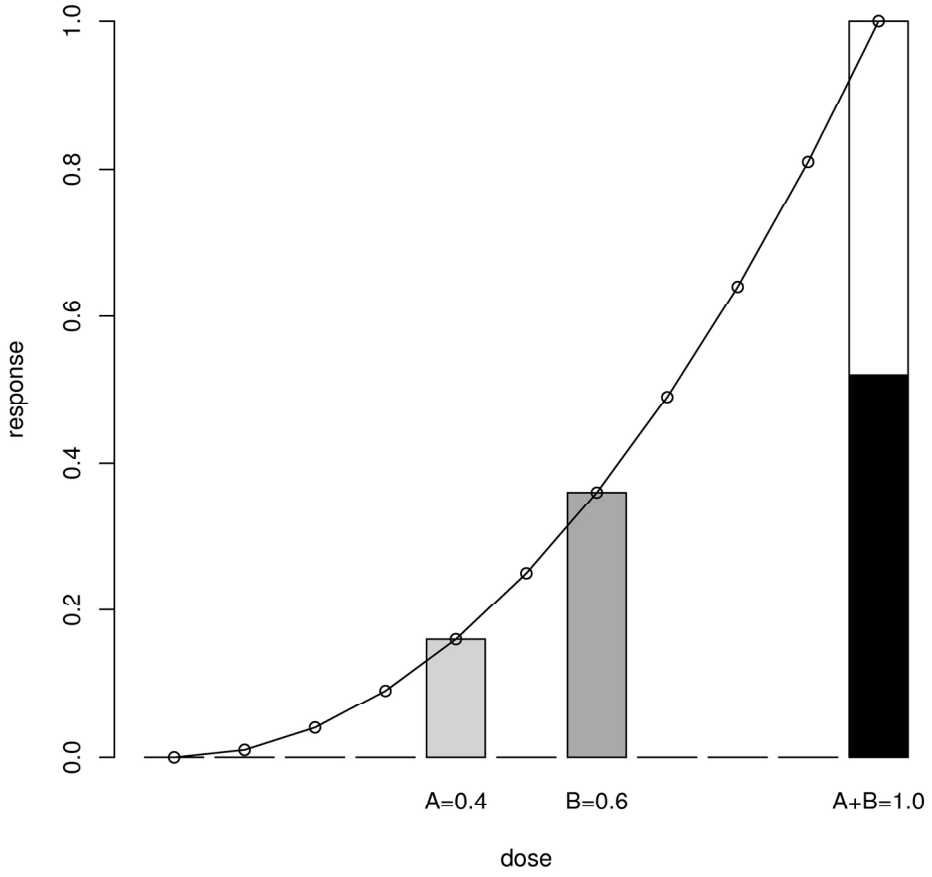

Figure 1. The "sham combination" of two identical agents, in doses A=0.4 and B=0.6, yields a larger response than the sum of the individual effects if the dose-response curve has increasing slope.
177x177mm (300 x 300 DPI)

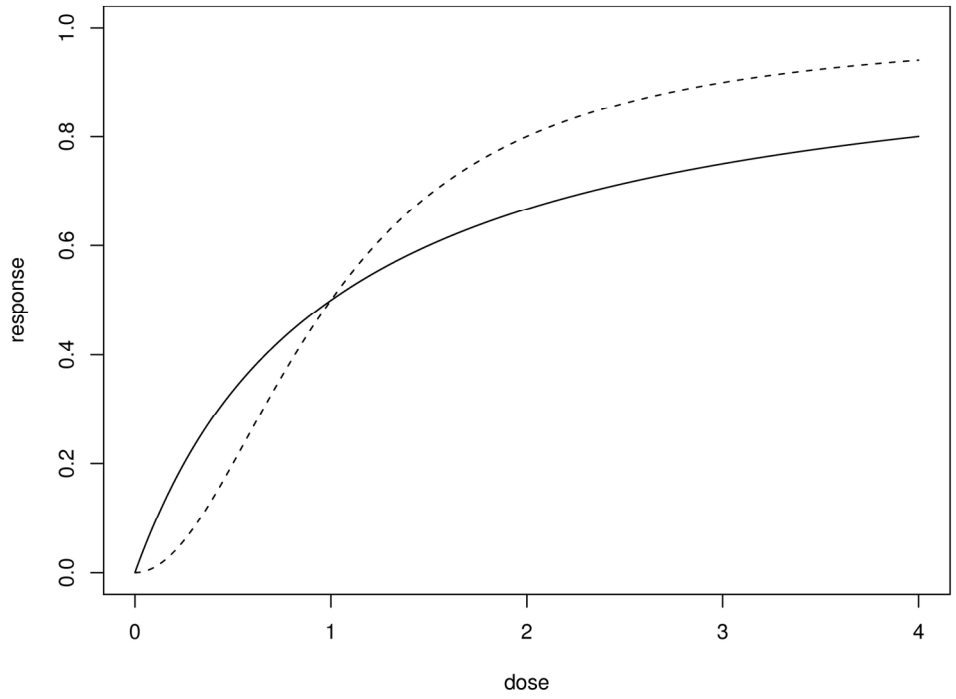

Figure 2. Hill functions with slope parameters n=1 (solid line) and n=2 (dashed line). In each case, K=1.
166x136mm (300 x 300 DPI)

Figure 3. Responses to the *n*=1 TEF joint exposure.

(a) Continuous response

dose of B \ dose of A	0	10.0
0	0	0.833
6.0	0.857	0.917

(b) Risk (threshold of 0.8)

	dose of A	
	0	10.0
0	0	1
6.0	1	1

Figure 4. Responses to the *n*=2 TEF joint exposure

(a) Continuous response

dose of B \ dose of A	0	0.3
0	0	0.022
0.2	0.038	0.109

(b) Risk (threshold of 0.1)

	dose of A	
	0	0.3
0	0	0
0.2	0	1